

Status of the Claims

Following entry of the amendments claims 29-41 and 43-57 will be pending in the application.

Summary of the Amendments

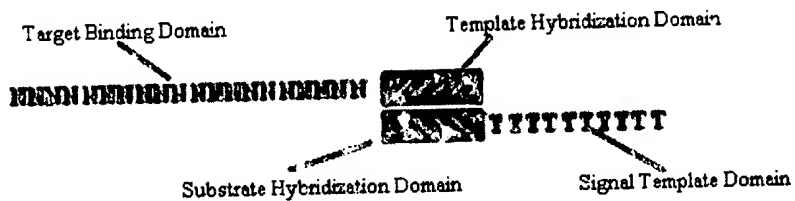
Claim 29 has been amended to clarify that the Substrate and Template Hybridization Domains "consist of sequences of less than 10 nucleotides" and that the addition of labeled nucleotides by DNA polymerase creates an oligonucleotide having from 5' to 3' an unlabeled Target Binding Domain, a Template Hybridization Domain, and a labeled Signal Domain. Claim 51 has been amended to clarify that the first and second nucleic acids hybridize to each other under conditions in which an enzyme can extend the second nucleic acid. Claim 57 has been added.

Summary of the Invention

The invention is for a general method of labeling the 3' end of oligonucleotides with a defined number of detectable markers. The method produces a more uniform labeled oligonucleotide product and several-fold higher levels of marker incorporation than has previously been available.

The method involves annealing two oligonucleotides together through short hybridization domains that consist of less than 10 nucleotides. The oligonucleotides include a hybridization probe (termed a "Substrate Nucleic Acid" in the application) and a template (termed a "Template Nucleic Acid"). The template defines which nucleotides to add to the hybridization probe and the number of additions. The hybridization probe contains a short sequence of less than 10 nucleotides, such as GGCGGG, added toward its 3'-end (termed a "Template Hybridization Domain") to facilitate hybridization with the template through the template's hybridization domain (termed a "Substrate Hybridization Domain"). The region of the template oligonucleotide that serves as a template for the addition of nucleotides containing the detectable marker (i.e. α -³²P phosphate) to the 3'-end of the hybridization probe is usually a sequence of T nucleotides. This region is designated as the "Signal Template Domain." Following the annealing step, nucleotides containing detectable labels or markers are incorporated onto the 3' end of the hybridization probe using a DNA polymerase, such as the Klenow fragment. The terminology and method are diagramed below.

In re Appln. of Behlke et al.
Application No. 09/497,943



Labeling Reaction, add DNA polymerase, alpha-32P-ATP





In re Appln. of Behlke et al.
Application No. 09/497,943

COPY OF PAPERS
ORIGINALLY FILED

REMARKS

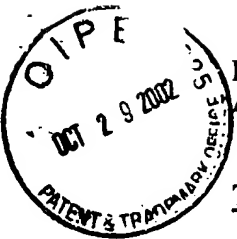
The Rejection Under 35 U.S.C. § 112, Second Paragraph

Claims 51-54 were rejected under 35 USC 112, first paragraph as containing subject matter which allegedly was not described in the specification in such a way as to reasonably convey to one of skill in the art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The examiner indicated that the invention of claims 51-54 was for a kit which comprised various nucleic acid sequences. The examiner took the position that the claims did not define in terms of what they were other than to indicate that they were comprised of a range of nucleotides and that the specification does not provide an adequate written description of the genus of nucleic acids that are now claimed. For support the examiner relied upon *Enzo Biochem Inc. v. Gen-Probe Inc.* (CAFC 01-1230, April 2002). With respect to the possession test under *Enzo*, the examiner did not find from the disclosure that the applicant was in possession of the claimed nucleic acid sequences, citing *Lockwood v. American Airlines, Inc.* (Fed. Cir. 1997) 41 USPQ2d 1961, 1966.

As a preliminary matter the applicant notes that after the Office Action was issued, the Federal Circuit reversed its decision in *Enzo* as an incorrect statement of law. Therefore, to the extent that the decision supported the present rejection, that basis of support is now absent.

The applicant requests the Examiner to reconsider and withdraw this rejection for the following reasons. Claims 51-54 have been amended to more clearly define the genus of oligonucleotides encompassed by the claims. Specifically, claims 51-54 now require that the Substrate and Template Hybridization Domains "consist of a sequence of less than 10 nucleotides" and "the hybridization domains of the first and second nucleic acids hybridize to each other under conditions in which an enzyme can extend the second nucleic acid by adding a sequence complementary to the Substrate Template Domain." The applicant submits that amended claims 51-54 meet the written description requirement because they fully describe the genus of the oligonucleotides in the kit. Specifically, the oligonucleotides of the kit are required to contain sequences of less than 10 nucleotides that can anneal under suitable conditions for a primer extension reaction to occur. One of skill in the art would know whether the two such oligonucleotide sequences were hybridizable under these conditions and would therefore understand the metes and bounds of the claimed subject matter.

Moreover, disclosure that provides additional incite into the meaning of these claim limitations can be found in the specification at page 7, line 31 - page 9, line 6; page 10, lines 5 - 36; page 12, lines 12 - 15; page 20 line 3 - page 22, line 29 and elsewhere in the application. In addition, diagrams at Fig. 1, Fig. 2A, Fig. 4A, Fig. 5A, Fig. 6A demonstrate oligonucleotides that can be used in the kit. The sequence listings SEQ ID NOS 1-3 provide exemplary hybridization sequences for use in oligonucleotides of the invention however; one of skill could easily identify additional workable sequences. The applicant submits that claims 51-54 now meet the written description requirement and are allowable.



The Rejection Under 35 U.S.C. § 102(e)

Claims 29, 30, 32, 33, 40 and 44 remain rejected under 35 U.S.C. 102(e) as being anticipated by Shuber. The rejection was based on Shuber at column 2, third paragraph and column 4, third paragraph which discloses the use of a chimeric primer that is described as being configured 5'-XY-3'. The examiner indicated that the "X" domain "comprises a sequence that does not hybridize to the target sequence or its complement." Therefore, the "X" domain was considered to meet the applicant's limitation of a "Signal Template Domain" and the "Y" domain was considered to meet the applicant's limitation of a "Substrate Hybridization Domain" of the "first sequence." The examiner stated that target sequence of Shuber met the limitations of applicant's second sequence because at column 4 of Shuber the respective domains could comprise nearly any nucleotide sequence and range in length from 17 to 25 bases. This disclosure in Shuber was considered to meet the limitation of applicant's claim 34 requiring a Substrate Hybridization Domain that "comprises a sequence of about 5 to 10 nucleotides."

The examiner indicated that neither of applicant's sequences were defined in terms of primer or template but were defined as having a sequence length of about 105 to about 120 nucleotides (applicant's first sequence) and about 5 to 20 nucleotides (applicant's second sequence) and additional nucleotides could be included in those sequences. With respect to claim 29, the first independent claim, the examiner noted that there was a protruding end for both the first and second sequences and that both sequences could be extended such that both strands could serve as either primer or template. Therefore, applicant's prior argument was not deemed persuasive.

The applicant respectfully requests the examiner to reconsider and withdraw this rejection for the following reasons. As amended, claim 29 now requires that the Substrate and Template Hybridization Domain "consist[s] of a sequence of less than 10 nucleotides." As the examiner pointed out in the Office Action, Shuber discloses that the corresponding region can be from 17 to 25 bases. Thus, amended claim 29 clearly distinguishes over Shuber.

Claim 29 distinguishes over Shuber in another way. Specifically, claim 29 requires "extending the second nucleic acid with a DNA polymerase in the presence of a labeled nucleotide to create a labeled Signal Domain. Shuber fails to disclose extending a nucleic acid with a labeled nucleotide to create a labeled signal domain as required by claim 29. The reason for this is that Shuber is directed to multiplex DNA amplification methods and the resulting amplified target sequences are the entities to be detected rather than for detecting other sequences. According to Shuber, the amplified products can be detected by hybridization with allele specific oligonucleotides, by restriction endonuclease cleavage, or by single stranded conformational polymorphism (SSCP) analysis or visualization on agarose gels. See column 2, lines 40-44.

The applicant submits that because Shuber fails to disclose or provide an incentive to label its extension products or to generate oligonucleotides with hybridization domains of less than 10 nucleotides, Shuber does not anticipate or make claim 29 obvious. Likewise, rejected claims 30, 32, 33, 40, and 44 which depend from claims 29 and therefore contain all of its limitations are not anticipated or made obvious for the same reasons.



In re Appln. of Behlke et al.
Application No. 09/497,943

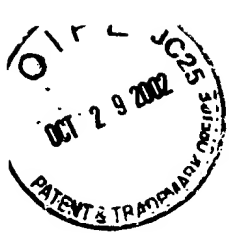
The Rejections Under 35 U.S.C. § 103(a)

Claim 31 was rejected as obvious under 35 U.S.C. 103(a) as being unpatentable over Shuber in view of Khan et al. The examiner indicated that Khan et al. discloses that suitable templates include DNA as well as RNA at column 8. The examiner took the position that it would have been obvious to one of ordinary skill in the art to have modified the method of Shuber whereby RNA was used as a template and to have used a RNA dependent DNA polymerase so as to generate cDNA runoffs that would resist degradation and provide a more stable resource for further investigation. The examiner took the position that the art is well developed in this area so that a skilled artisan would have a reasonable expectation of success.

The applicant requests the examiner to reconsider and withdraw this rejection for the following reason. As discussed above, the applicant submits that claim 29 distinguishes over Shuber by requiring that the Substrate and Template Hybridization Domain "consist[s] of a sequence of less than 10 nucleotides" because Shuber's primers, which are for multiplex PCR, have hybridization domains that are 17-25 nucleotides in length. Khan, which is primarily directed to a new propargylethoxyamino nucleotide compound and its use in sequencing and PCR reactions, only discloses primers of 21 (*see e.g.*, column 12, line 25; column 13, line 32) and 25 nucleotides in length (*see e.g.*, column 15, line 32), all of which hybridize along their entire length. Thus, neither Shuber or Khan disclose a set of hybridizing oligonucleotides that have hybridization domains that consist of a sequence of less than 10 nucleotides as required by claim 29. Consequently, Shuber and Khan cannot be combined to provide all of the limitations of claim 29. Nor do these references, taken separately or collectively, provide an incentive to use hybridization domains of less than 10 nucleotides because both patents are directed to PCR and DNA sequencing reactions which are known to require longer hybridization domains to ensure unique and specific priming of these reactions. Claim 31 which depends from claim 29 contains all of its limitations and is patentable over Shuber and Khan for the same reasons. Therefore, the applicant respectfully requests that this rejection be withdrawn.

16. Claims 35-38, 41-43 and 48 were rejected under 35 U.S.C. 103(a) as unpatentable over Shuber in view of Grossman et al. and Khan et al. The examiner noted that Shuber does not disclose homopolymeric tails but that Grossman et al., column 19, discloses the generation of homopolymeric tails and the use of a detectable nucleotide, e.g., a fluorophore (applicant's fluorescein) and Khan discloses labels that can be used in primer extension reactions and discloses the use of modified nucleotides, e.g., dideoxynucleotides, which proscribe primer elongation at column 5, sixth paragraph. Therefore, the examiner rejected the claims, concluding that it would have been obvious to incorporate any one of the labels disclosed by Khan et al., and Grossman et al., into either strand or into a primer extension product of Shuber. The examiner concluded that it would have been obvious to incorporate chain terminating nucleotides into sequences to limit the amount and direction of an extension reaction.

The applicant submits that the homopolymeric tailing method of Grossman is quite different from the extension method of claims 35-38, 41-43 and 48. The "homopolymeric tailing" method referred to by Grossman is well known in the art to involve extending the 3'-



In re Appln. of Behlke et al.
Application No. 09/497,943

end of an oligonucleotide (in the absence of an annealed complementary sequence) with nucleotides using the enzyme Terminal Deoxynucleotidyl Transferase (TdT). In contrast, the present invention requires extending an oligonucleotide with a DNA polymerase in the presence of an annealed oligonucleotide having a Signal Template Domain so as to create a "labeled Signal Domain having a sequence which shows complementarity toward and is hybridizable to the Signal Template Domain."

This difference is not trivial. The Terminal Deoxynucleotidyl Transferase adds variable numbers of nucleotides to the 3'-ends of oligonucleotides independently of the complementary template required for DNA polymerase activity and by the present invention. Consequently, the TdT derived product contains substantially greater variability in its chemical makeup that can range from unlabeled to hundred-fold labeled species. This makes it difficult to produce and use a TdT probe in a reproducible manner.

Grossman also fails to remedy the other deficiencies of Shuber when combined with Khan as set out above. Neither Shuber, nor Khan, nor Grossman disclose hybridization domains that consist of less than 10 nucleotides as required by all of the now pending claims. Thus, even if there were a motivation to make the combination of the three cited references proper, which applicant disputes, the combination would lack Substrate and Template Hybridization Domains that "consist of a sequence of less than 10 nucleotides." Nor does the disclosure of Grossman provide any motivation over Shuber and Khan (which lack any such incentive) to provide such hybridization domains. Because each of the rejected claims requires Substrate and Template Hybridization Domains that "consist of a sequence of less than 10 nucleotides" by virtue of their dependence on claim 29, and because Grossman's homopolymeric tailing method is completely different than the method in applicant's claims, the applicant requests the examiner to reconsider and withdraw this rejection.

Claims 45-47 were rejected under 35 U.S.C. 103(a) as being unpatentable over Shuber in view of Brown. The examiner indicated that Shuber does not disclose the use of 2,6-diaminopurine, but that Brown discloses the use of that compound in oligonucleotides and their use in primer extension reactions. It would have been obvious to one of skill in the art to have incorporated the use of 2,6-diaminopurine into the sequences utilized by Shuber for the obvious improvements as disclosed by Brown.

As with the other references cited by the examiner, the applicant submits that Brown fails to disclose hybridization domains that contain less than 10 nucleotides as required by the rejected claims. Brown indicates that representative oligonucleotide sequences are given in Figures 5-9 at page 8, 3rd complete paragraph which figures show oligonucleotides that are all 17 nucleotides in length. Thus, the combination of Brown with Shuber would not contain all of the limitations of the rejected claims, namely Template and Substrate Hybridization Domains that consist of a sequence of less than 10 nucleotides. Moreover, as with Shuber, the primer extension reaction referred to by Brown is for DNA sequencing or PCR amplification reactions in which oligonucleotide hybridization occurs over a considerably longer stretch of nucleic acids than required by the rejected claims. Therefore, the applicant



In re Appln. of Behlke et al.
Application No. 09/497,943

COPY OF PAGES
ORIGINALLY FILED

submits that Brown provides no incentive to provide hybridization domains of less than 10 nucleotides and does not make the invention of claims 45-47 obvious. Consequently, the applicant kindly requests the Examiner to reconsider and withdraw this rejection.

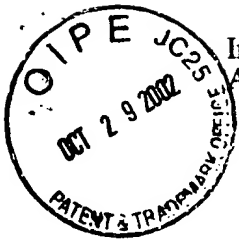
Conclusion

The application is considered in good and proper form for allowance, and the examiner is respectfully requested to pass this application to issue. If, in the opinion of the examiner, a telephone conference would expedite the prosecution of the subject application, the examiner is invited to call the undersigned attorney.

Respectfully submitted,

Robert M. Gould
Reg. No. 43,642
Vice President of Intellectual Property
Integrated DNA Technologies, Inc.
8930 Gross Point Rd.
Suite 700
Skokie, Illinois 60077

Date: October 23, 2002



In re Appln. of Behlke et al.
Application No. 09/497,943

CERTIFICATE OF MAILING

I hereby certify that this Amendment and the documents referenced as being concurrently submitted are being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to:

Assistant Commissioner for Patents
Washington, DC. 20231

on October 23, 2002.

Typed or printed name of person signing this certificate:

Robert Gould

Signature

A handwritten signature of Robert Gould in cursive script, written over a horizontal line.



COPY OF PAPERS
ORIGINALLY FILED

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Behlke et al.

Art Unit: 1634

Application No. 09/497,943

Examiner: Bradley L. Sisson

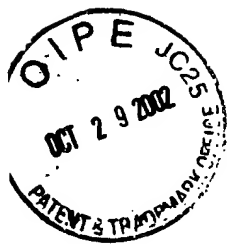
Filed: February 4, 2000

For: Primer extension methods for
production of high specific activity
nucleic acid probes

AMENDMENTS TO CLAIMS MADE IN RESPONSE TO
OFFICE ACTION DATED APRIL 23, 2002

Amendments to existing claims:

29. A method of labeling a nucleic acid molecule, comprising the steps of:
- a. Hybridizing a first nucleic acid to a second nucleic acid, wherein the first nucleic acid comprises, from 3' to 5': a Substrate Hybridization Domain and a Signal Template Domain, wherein:
 - i. the Substrate Hybridization Domain [comprises] consists of a sequence of [about 5 to about 20] less than 10 nucleotides; and
 - ii. the Signal Template Domain [comprises] consists of a sequence of [about 5 to about 20] less than 10 nucleotides;and the second nucleic acid comprises from 3' to 5': a Template Hybridization Domain and a Target Binding Domain, wherein:
 - i. the Template Hybridization Domain comprises a sequence of about 5 to about 20 nucleotides, is not detectably labeled, and shows complementarity toward and is hybridizable to the Substrate Hybridization Domain of the first nucleic acid;
 - ii. the Target Binding Domain is not detectably labeled and comprises a nucleotide sequence heterologous to that of the Template Hybridization Domain;
- and:
- b. extending the second nucleic acid with a DNA polymerase in the presence of a labeled nucleotide to create an oligonucleotide having from 5' to 3' an unlabeled Target Binding Domain, a Template Hybridization Domain, and a labeled Signal Domain having a sequence which shows complementarity toward and is hybridizable to the Signal Template Domain., thereby labeling said second nucleic acid molecule.]
30. The method of claim 29, wherein the nucleotides which comprise the first or second nucleic acid are deoxyribonucleotides.



In re Appln. of Behlke et al.
Application No. 09/497,943

31. The method of claim 29, wherein the nucleotides which comprise the first or second nucleic acid are ribonucleotides.
32. The method of claim 29, wherein the second nucleic acid consists of about 15 to about 150 nucleotides.
33. The method of claim 29, wherein the Substrate Hybridization Domain is at the 3' end of the first nucleic acid.
34. The method of claim 29, wherein the Substrate Hybridization Domain [comprises] consists of a sequence of about 5 to [about] less than 10 nucleotides.
35. The method of claim 29, wherein the Substrate Hybridization Domain cannot be extended by a 5'→3' DNA polymerase.
36. The method of claim 35, wherein the Substrate Hybridization Domain further comprises an extension of nucleotides at the 3' end of said Substrate Hybridization Domain, the extension having no complementarity to the Template Hybridization Domain of the second nucleic acid.
37. The method of claim 35, wherein the Substrate Hybridization Domain comprises a 3'-terminal modified nucleotide.
38. The method of claim 37, wherein the modification is selected from the group consisting of: a 3'-amino-modifier, a 2', 3'-dideoxynucleotide, a 3'-phosphate, and a modified 3'-phosphate group.
39. The method of claim 37, wherein the Substrate Hybridization Domain comprises at least one nucleotide which comprises a modified cytidine, which nucleotide is selected from the group consisting of: C5-methyl-dC and C5-propynyl-dC.
40. The method of claim 29, wherein the Signal Template Domain comprises a sequence of about 10 to about 50 nucleotides.
41. The method of claim 29, wherein the Signal Domain is at least 50%, at least 70%, at least 90% or 100% homopolymeric.
42. [deleted]
43. The method of claim 29, wherein at least 60% of the nucleotides of the Template Hybridization Domain comprise guanosine or cytidine or a combination thereof, and the Signal Domain is at least 50% homopolymeric.
44. The method of claim 29, wherein the extending step is carried out by a DNA polymerase selected from the group consisting of: *E. coli* DNA polymerase I holoenzyme, Klenow fragment of *E. coli* DNA polymerase I, T4 DNA polymerase, T7 DNA polymerase, and a DNA polymerase encoded by a thermophilic bacterium.

In re Appln. of Behlke et al.
Application No. 09/497,943

45. The method of claim 29, wherein the Template Hybridization Domain or the Substrate Hybridization Domain comprises at least one modified nucleotide, which modified nucleotide increases the hybridization affinity of said Template Hybridization Domain to said Substrate Hybridization Domain.

46. The method of claim 45, wherein at least one modified nucleotide is found in the Template Hybridization Domain.

47. The method of claim 46, wherein at least one modified nucleotide is selected from the group consisting of: C5-methyl-dC, C5-propynyl-dC, C5-propynyl-dU, and 2,6-diaminopurine.

48. The method of claim 29, wherein at least one nucleotide comprises a label selected from the group consisting of: ^{32}P , ^{33}P , ^{35}S , fluorescein, digoxigenin, biotin, Cy5, Cy3, and rhodamine.

49. (amended) A method for detecting a Target Nucleic Acid in a sample, comprising:

- a. contacting the sample with a Complex under conditions whereby said Complex can bind to the Target Nucleic Acid to form a Complex-Target Nucleic Acid hybrid; wherein said complex comprises:
 - i. a first nucleic acid comprising, from 3' to 5': a Substrate Hybridization Domain and a Signal Template Domain, wherein:
 - (1) the Substrate Hybridization Domain comprises a sequence of about 5 to about 20 nucleotides; and
 - (2) the Signal Template Domain comprises a sequence of about 5 to about 100 nucleotides;and
 - ii. a second nucleic acid comprising from 3' to 5': a Signal Domain, a Template Hybridization Domain and a Target Binding Domain, wherein:
 - (1) the Signal Domain comprises a sequence of about 5 to about 100 nucleotides, which sequence shows complementarity toward and is hybridizable to the Signal Template Domain of the first nucleic acid, and of which at least two nucleotides are detectably labeled;
 - (2) the Template Hybridization Domain comprises a sequence of about 5 to about 20 nucleotides, is not detectably labeled, and shows complementarity toward and is hybridizable to the Substrate Hybridization Domain of the first nucleic acid;
 - (3) the Target Binding Domain is not detectably labeled and comprises a nucleotide sequence heterologous to that of the Template Hybridization Domain;and

- b. detecting any Complex-Target Nucleic Acid hybrids, so that if a Complex-Target Nucleic Acid hybrid is detected, a Target Nucleic Acid is detected in the sample.

50. (amended) A method for detecting a Target Nucleic Acid in a sample, comprising:
- a. dissociating a Complex to generate a single stranded first nucleic acid and a single stranded second nucleic acid; wherein said complex comprises:
 - i. a first nucleic acid comprising, from 3' to 5': a Substrate Hybridization Domain and a Signal Template Domain, wherein:
 - (1) the Substrate Hybridization Domain comprises a sequence of about 5 to about 20 nucleotides; and
 - (2) the Signal Template Domain comprises a sequence of about 5 to about 100 nucleotides;
 - and
 - ii. a second nucleic acid comprising from 3' to 5': a Signal Domain, a Template Hybridization Domain and a Target Binding Domain, wherein:
 - (1) the Signal Domain comprises a sequence of about 5 to about 100 nucleotides, which sequence shows complementarity toward and is hybridizable to the Signal Template Domain of the first nucleic acid, and of which at least two nucleotides are detectably labeled;
 - (2) the Template Hybridization Domain comprises a sequence of about 5 to about 20 nucleotides, is not detectably labeled, and shows complementarity toward and is hybridizable to the Substrate Hybridization Domain of the first nucleic acid;
 - (3) the Target Binding Domain is not detectably labeled and comprises a nucleotide sequence heterologous to that of the Template Hybridization Domain;
 - b. contacting the sample with the second nucleic acid of step a. under conditions whereby said second nucleic acid can bind to the Target Nucleic Acid to form a second nucleic acid-Target Nucleic Acid hybrid; and
 - c. detecting any second nucleic acid-Target Nucleic Acid hybrids, so that if a second nucleic acid-Target Nucleic Acid hybrid is detected, a Target Nucleic Acid is detected in the sample.

51. A kit for labeling a nucleic acid molecule, comprising a reaction mixture and a DNA polymerase, wherein the reaction mixture comprises:

- a. a first nucleic acid comprising, from 3' to 5': a Substrate Hybridization Domain and a Signal Template Domain, wherein:
 - i. the Substrate Hybridization Domain comprises a sequence of about 5 to about 20 nucleotides; and
 - ii. the Signal Template Domain comprises a sequence of about 5 to about 100 nucleotides;
- and

- b. a second nucleic acid comprising from 3' to 5': a Template Hybridization Domain and a Target Binding Domain, wherein:
 - i. the Template Hybridization Domain [comprises] consists of a sequence of [about 5 to about 20] less than 10 nucleotides, is not detectably labeled, and shows complementarity toward and is hybridizable to the Substrate Hybridization Domain of the first nucleic acid;
 - ii. the Target Binding Domain is not detectably labeled and comprises a nucleotide sequence heterologous to that of the Template Hybridization Domain[.];
- c. wherein the hybridization domains of the first and second nucleic acids hybridize to each other under conditions in which an enzyme can extend the second nucleic acid by adding a sequence complementary to the Signal Template Domain.

52. The kit of claim 51, wherein at least 60% of the nucleotides of the Template Hybridization Domain comprise guanosine or cytidine or a combination thereof, and the Signal Domain is at least 50% homopolymeric.

53. The kit of claim 51, wherein the Substrate Hybridization Domain comprises a predetermined sequence comprising CCCGCC and the Signal Template Domain comprises a predetermined sequence comprising TTTTTTTTTT.

54. The kit of claim 51, wherein, the first nucleic acid comprises a predetermined sequence comprising SEQ ID NO:10.

55. The method of claim 29, wherein the Probe has a specific activity of at least 7×10^7 CPM per picomole, and wherein the Probe comprises the Target Binding Domain, the Template Hybridization Domain and the Signal Domain.

56. The method of claim 29, wherein the Probe has a specific activity of at least 9×10^7 CPM per picomole, and wherein the Probe comprises the Target Binding Domain, the Template Hybridization Domain and the Signal Domain.

57. The method of claim 29, wherein the first nucleic acid has a hairpin loop disposed to the 5' side of the Signal Template Domain.